In the United States Patent and Trademark Office

IN RE APPLICATION OF:
Wilhelm Rademacher et al.
US Serial No. 10/578,333
FOR: Mixtures comprising Strobilurines and Ethylene Modulators

DECLARATION

I, Lutz Brahm, Dr. agr., citizen of the Federal Republic of Germany and residing at: Am Hang 16, 67551 Worms, Germany, hereby declare as follows:

I am a fully trained Agronomist having studied Agriculture at the Justus-Liebig-University of Giessen, Germany, from 1987 to 1993. I received a Diploma Degree in 1993 by the Justus-Liebig-University of Giessen, Germany. In 1997, I received the doctorate degree (Ph.D.) by the Justus-Liebig-University of Giessen, Germany.

I joined BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany, in 2006. Since then, I have been working in the field of crop protection;

The tests were carried out under my supervision in accordance with the instructions given in thee specification of Appl. Ser. No. 10/578,333 or as described below.

Soybean plants (Pioneer 9091) were grown in pots in 2010 in the greenhouse at the BASF agricultural center at Limburgerhof, Germany. The trial was setup with 10 replications with one pot 1 plant each per replication.

The active ingredients were used as formulations. The formulations were used in the dose rates given in Table 1. The products were applied in a total spray volume of 400 l/ha. Products were diluted in water. The spray solution was applied in a spray cabinet using a spray boom with flat fan nozzles.

Prohexadion-Ca was applied as REGALIS (WG, 10% active ingredient per liter) at soybean growth stage 12 (BBCH). Pyraclostrobin was applied at growth stage 12 (BBCH) as HEADLINE™ (EC, 250 g active per liter). Mixtures tested in the experiment were applied as tank mixes.

Total fresh shoot biomass of the single plants was harvested 7 days after treatment. Plants were stressed by placing the cut shoots in a dryer cabinet for 25-30 minutes at 30-35°C. After the stress treatment weight of the single plants was recorded and water loss was calculated as the difference of fresh weight and weight after the stress treatment to insure that all treatments faced the same stress level. Plants were then placed in glass bottles which were sealed with a rubber septum.
Twenty two and forty four hours after stress treatment a sample of the headspace of each glass bottle was taken and injected into a gas chromatograph to measure the ethylene content in the headspace of the bottles produced by the stressed soybean plants. Ethylene production of the plants was calculated as the ethylene production per g of plant material after stress treatment. Data is given in Table 1.
The efficacy of the products and the mixtures in reducing ethylene production was calculated as % decrease of ethylene produced per g of shoot biomass after the stress treatment compared to the untreated control:

$$E = a/b\text{-}1 \cdot 100$$

a corresponds to ethylene produced per g biomass after stress treatment in the treated plants in ppm and
b corresponds ethylene produced per g biomass after stress treatment in the untreated (control) plants in ppm.

An efficacy of 0 means the ethylene produced by the treated plants corresponds to that of the untreated control plants; an efficacy of 100 means the treated plants showed a decrease in ethylene production of 100% (no more ethylene produced). An efficacy of -100 means the ethylene production was increased by 100%.

The expected efficacies of the combinations of the active compounds were estimated using Colby´s formula (Colby, S.R., Calculating synergistic and antagonistic responses of herbicide combinations, Weeds, 15, pp. 20-22, 1967) and compared with the observed efficacies.

Colby´s formula: $E = x + y - x \cdot y/100$

E expected efficacy, expressed in % of the untreated control, when using the mixture of the active compounds A and B at the concentrations a and b
x efficacy, expressed in % of the untreated control, when using the active ingredient A at the concentration a
y efficacy, expressed in % of the untreated control, when using the active ingredient B at the concentration b

Table 1: Effect of prohexadion-Ca and pyraclostrobin application at growth stage 12 (BBCH) on on stress related ethylene production of soybean plants. Negative values for ethylene reduction means more ethylene than in untreated is produced.

Treatment	AI rate [g/ha]	Ethylene reduction: Observed Efficacy [%]		Ethylene reduction Expected Efficacy* [%]	
		22h	44h	22h	44h
Pyraclostrobin	25	-1,7	-19,7		
Prohexadion-Ca	25	3,2	-1,0		
Pyraclostrobin + Prohexadion-Ca	25 25	12,8	28,8	-17,3	-33,5

* according to Colby's formula
# expected biomass increase according to Colby´s formula

The results demonstrate that the efficacy of the combinations of the active compounds to reduce ethylene production shown in table 1 is higher at 22 or 44 hours (table 1) after treatment than the expected efficacy calculated using Colby's formula.

The test results show that the observed ethylene reduction was higher than the expected ethylene reduction calculated using Colby's formula, i.e., the active ingredients interacted synergistically when combined with one another.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Limburgerhof, 25th of October, 2010

(Signature of Declarant)